

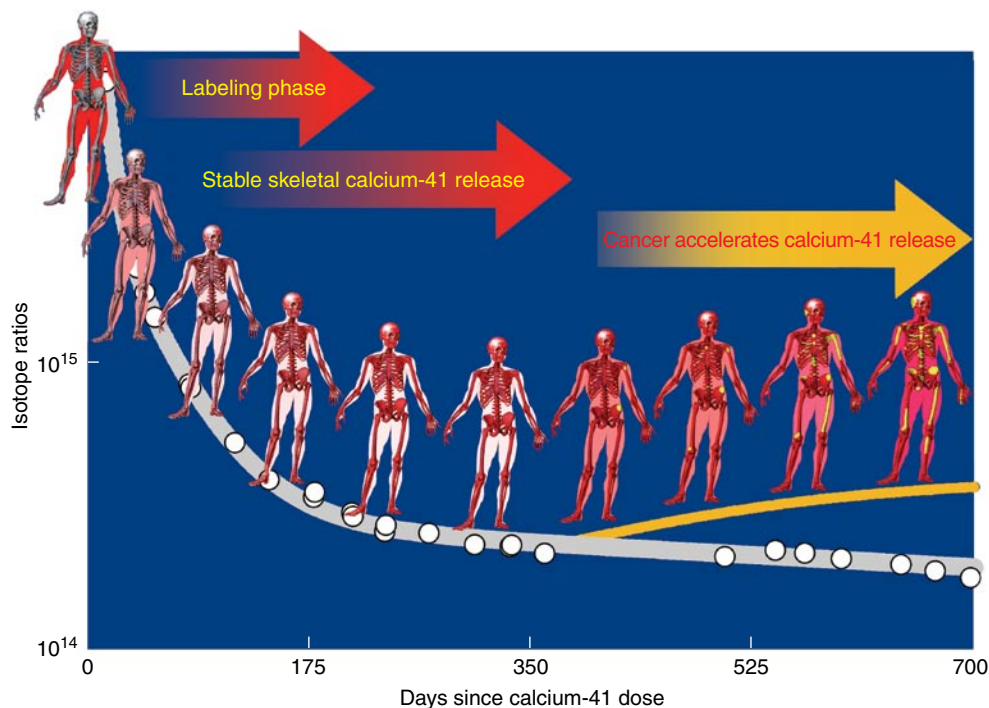
Early Detection of Bone Disease

THE American Cancer Society estimates that more than 230,000 new cases of prostate cancer and 212,000 of breast cancer will be diagnosed in the U.S. in 2006. In at least 80 percent of the cases that result in death, tumors will have spread to bone and significantly damaged the skeleton. This damage often causes uncontrollable pain and usually goes undetected until it is too late to treat effectively. Bone cancer spreads quickly because the skeleton is a nutrient-rich environment for the malignant cells. As tumor cells multiply, they also send signals to the body that trigger a rapid increase in bone destruction.

Medical researchers know that skeletal disease correlates with the bone turnover rate. The skeleton is a dynamic system composed of protein and calcium minerals. At any one time, about 10 percent of the system is in the process of being formed or destroyed. In adult humans, the skeleton is completely replaced once every 10 to 15 years. Early detection of the increased bone destruction that occurs in the initial stages of cancer metastasis could help physicians design methods to prolong a patient's life or even arrest disease progression.

Conventional diagnostic methods are not sensitive enough to detect early-stage bone cancer. For example, measurements of the bone turnover "markers" found in blood or urine samples normally fluctuate by 20 to 30 percent, making small changes impossible to detect. Medical imaging methods can be sensitive to bone abnormalities. However, these diagnostics cannot be used for routine screening because the equipment is expensive to operate, the tests expose patients to radiation, and interpreting the results is a time-consuming process requiring highly skilled personnel.

A team of Livermore scientists, with funding from the Laboratory Directed Research and Development Program and the National Institutes of Health (NIH), has developed a technique that improves the diagnostic capabilities for bone disease. The technique uses accelerator mass spectrometry (AMS) with calcium-41 as an isotopic tracer to measure small changes in the rate of skeletal bone turnover. With this technique, physicians



Accelerator mass spectrometry (AMS) can measure a calcium-41 tracer in blood or urine to track small changes in skeletal calcium. After the tracer mixes with the body's calcium, the calcium levels stabilize, providing a baseline. Tracer concentration can then be measured over time. A sudden increase in the amount of calcium-41 released from bone (yellow line) signals the onset and progression of bone disease.

could monitor a patient's calcium level over his or her lifetime and detect metastatic cancer in the early stages, when treatment would be more effective. The AMS technique could also be used to diagnose other health problems involving calcium loss, such as osteoporosis and kidney failure.

High Precision with a Tiny Tracer

The Livermore team chose calcium-41 as the tracer isotope because only a small dose is needed for the diagnostic. Calcium-41 is extremely rare in nature, so its signal is clearly distinguished from those of other calcium isotopes that occur naturally in the body. In addition, calcium-41 has a long half-life (104,000 years) and decays by a low-energy process to potassium-41, a naturally occurring and stable isotope. "The dose to be given is less than one-fiftieth of the radiation a person would receive from a single x-ray bone-density test, but

this small amount of calcium-41 is enough to track the skeletal calcium loss over a person's lifetime," says team leader Darren Hillegonds, a chemist in the Laboratory's Center for Accelerator Mass Spectrometry (CAMS).

The amount of calcium-41 used in this research is so low that conventional mass spectrometry cannot separate it from other elements. AMS, however, is about a million times more sensitive than the conventional technique, so it can measure nanogram quantities of isotopes.

Livermore has used AMS to analyze isotopes for many research areas, including environmental and earth sciences, energy, materials analysis, and archaeological radiocarbon dating. In the early 1990s, the Laboratory pioneered the use of AMS for biomedical applications. In 1999, NIH designated CAMS as its National Research Resource for biomedical AMS. (See *S&TR*, November 1997, pp. 4–11; July/August 2000, pp. 12–19.)

The Livermore team is working with collaborators from the University of California (UC) at San Diego and the UC Davis Cancer Center, which sees more than 250 new breast cancer and 150 new prostate cancer patients each year. CAMS director John Knezovich says, "The calcium-41 work represents an exciting intersection of nuclear physics and health research. AMS enables scientists to begin to answer questions in biomedical research that cannot be studied anywhere else."

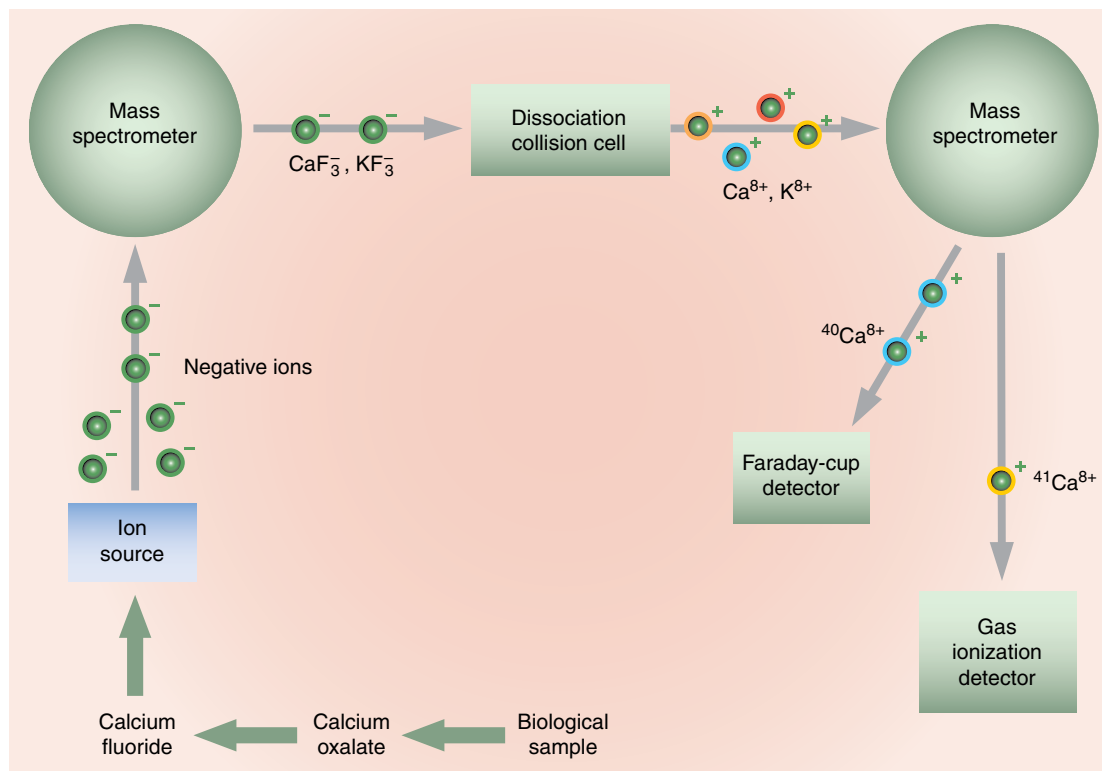
Detects Small Changes over a Lifetime

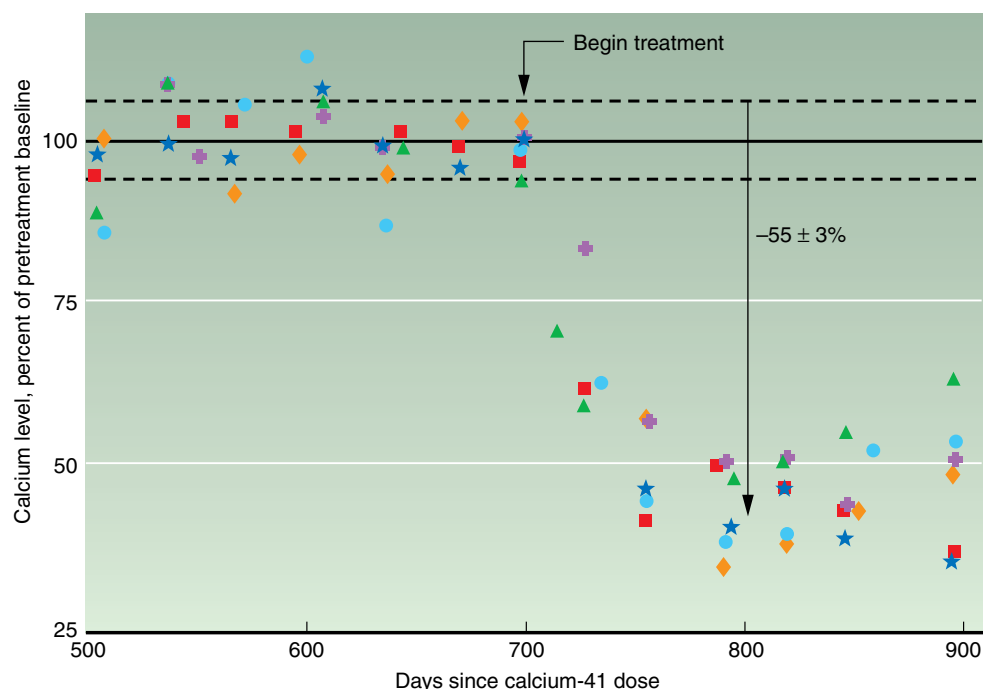
Hillegonds's team used UC Berkeley's Madonna software to develop a kinetic model of human calcium homeostasis. The model predicts how the levels of calcium-41 in urine will change during the first few hours after a dose is administered until many years later. The modeling results also helped validate the methods designed to administer a calcium-41 tracer to a patient and track changes in urine.

"Doctors would administer the tracer isotope to patients when they were diagnosed with breast or prostate cancer," says Hillegonds. "Within hours, the tracer would mix with the calcium in bodily fluids and tissues, and within months, it would be incorporated into the skeleton. The patient's calcium level could be monitored by testing urine samples several times a year. As bone breaks down, tiny amounts of calcium-41 atoms become markers in the urine and can be counted by AMS." If bone disease occurs, the calcium-41 level in a patient's urine would rise dramatically.

The Livermore researchers used a slow-neutron nuclear reactor and a naturally occurring calcium isotope to produce the calcium-41 tracer for their experiments. Collaborators at UC San Diego prepared the collected samples for analysis. AMS samples must be in solid form, but elemental calcium is unstable and difficult to produce. Therefore, the UC San Diego researchers mixed calcium from the samples with hydrofluoric acid to produce

The AMS process for analyzing calcium-41 begins with cesium ions producing negative calcium trifluoride (CaF_3^-) and potassium trifluoride (KF_3^-) ions. Because KF_3^- is less stable than CaF_3^- , this step effectively reduces the isotope potassium-41 (^{41}K) that otherwise would interfere with the analysis. A mass spectrometer separates these molecules, which accelerate and collide with a carbon foil. This collision destroys all of the molecules and leaves only positively charged particles, which are separated by a second mass filter. Each calcium 41 particle ($^{41}\text{Ca}^{8+}$) is positively identified and counted in a gas ionization detector, providing clear separation from the remaining $^{41}\text{K}^{8+}$. Stable calcium-40 particles ($^{40}\text{Ca}^{8+}$) are separately measured, and the ratio of the two calcium isotopes is compared to baseline levels.





Skeletal calcium was measured in six postmenopausal women who received a medication for osteoporosis. AMS measurements clearly show the expected drop in the rate of bone destruction for each individual tested, confirming that the drug is effective. Results also demonstrate that the calcium-41 technique is the most sensitive marker available to diagnose bone turnover.

a precipitate of calcium fluoride and added silver powder to the mixture so it would conduct a charge effectively.

With AMS, a beam of cesium ions bombards the calcium fluoride mixture, producing negatively charged calcium trifluoride. These molecules are attracted to a 9-megavolt charge in the linear accelerator and collide with a carbon foil, which causes them to dissociate into high-energy calcium particles. Large magnetic and electrostatic filters then separate the particles. The calcium-41 particles enter a gas ionization detector, where they are individually counted. The stable calcium-40 isotope is measured at the same time by a Faraday-cup detector. By working with ratios of calcium-41 to calcium-40, researchers can ensure that results are insensitive to changes in instrument parameters, especially negative-ion output among different samples. The ratio of the two isotopes is then compared to the baseline calcium level to determine the rate of calcium loss. The Livermore spectrometer can analyze about 100 samples of calcium-41 every day. Knezovich says, "No other instrument has the speed and throughput to analyze the thousands of samples necessary for calcium-41 studies of human health."

To validate the technique, the team injected prostate cancer cells from a human into the tibia of mice. As tumors grew and destroyed bone, the rate of calcium-41 release increased. Results from these experiments indicate that the technique can effectively diagnose changes in bone turnover with a precision of ± 3 percent.

The team will soon begin trial studies on humans using prostate cancer patients with and without confirmed bone metastasis. Patients will take a 1.2-microgram dose of calcium-41 and

provide 11 urine samples over a 6-month period. Hillegonds says, "Preliminary data and the kinetic model predict that our approach will detect a change of 10 percent in bone turnover. Such a small change would be completely invisible to other diagnostics."

Improving Therapeutic Drugs

The technique may also help pharmaceutical companies assess the effectiveness of drug treatments. For example, some medications designed to treat osteoporosis reduce bone breakdown by "sealing" the bone. Hillegonds's team confirmed that calcium-41 release from bone dropped dramatically when one type of medication was administered, indicating that the treatment was effective.

According to Hillegonds, the calcium-41 assay for prostate and breast cancer could easily be tailored for malignancies affecting other organs of the body as well as for diseases in which treatment increases bone loss. He adds that the collaboration with UC researchers allows the Laboratory to extend the reach of its innovations. "UC's expertise in clinical research and medicine enables us to apply our scientific breakthroughs toward helping improve the health of hundreds of thousands of people each year."

—Gabriele Rennie

Key Words: accelerator mass spectrometry (AMS), bone disease, calcium-41, cancer, Center for Accelerator Mass Spectrometry (CAMS).

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